Clin. exp. Immunol. (1973) **14,** 531–539.

DEFICIENCY OF THE SECOND COMPONENT OF COMPLEMENT ASSOCIATED WITH ANAPHYLACTOID PURPURA AND PRESENCE OF MYCOPLASMA IN THE SERUM

M. SUSSMAN,* J. H. JONES,† JUNE D. ALMEIDA‡ AND P. J. LACHMANN§

* *Department of Medical Microbiology, Welsh National School of Medicine, Cardiff;* † *Department of Medicine, Cardiff Royal Infirmary,* ‡ *Wellcome Research Laboratories, Beckenham, Kent, and* §*Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London*

(*Received* 27 *February* 1973)

SUMMARY

A patient, with deficiency of the second component of complement (C2), who has had anaphylactoid purpura for 12 years is described. Other classical complement components were present normally and the very markedly low total haemolytic complement activity was restored by the addition of functionally purified C2. Glycine-rich β-glycoprotein (GBG), measured antigenically, was reduced to about 50% of normal but the alternate pathway to complement activation was normal as judged by the platelet factor three activation test. Structures identified morphologically as mycoplasma were present in the patient's serum.

INTRODUCTION

Deficiency of the second component of complement (C2) has been described in five families (Silverstein, 1960; Klemperer, Woodworth, Rosen & Austen, 1966; Klemperer, Austen & Rosen, 1967; Cooper, Ten Bensel & Kohler, 1968; Ruddy *et al.*, 1970; Agnello, de Bracco & Kunkel, 1972). The serum of homozygously affected individuals showed a markedly depressed total haemolytic complement level, while immune adherence and bactericidal activity were relatively unaffected. Several cases have now been reported in which the C2 deficiency was associated with disease processes including systemic lupus erythematosus and glomerulonephritis (Agnello *et al.*, 1972; Pickering *et al.*, 1971; Ruddy *et al.*, 1972). We wish to describe a patient with C2 deficiency associated with an anaphylactoid purpuric rash.

Correspondence: Dr M. Sussman, Department of Medical Microbiology, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN.

CASE HISTORY

The patient (L.M.) was first admitted to hospital in January, 1960 when she was 10 years old. For 12 months she had complained of swelling of the knees and ankles and a purpuric eruption on the legs. She had also had vague abdominal pains but no vomiting or melaena. There was a raised, macular, erythematous rash with petechiae over the shins and dorsal aspects of the feet but there were no other abnormal physical signs.

Results of investigations were as follows. Haemoglobin 82%; white cell count 7000/mm^3; sedimentation rate 44 mm in 1 hr (maximum); platelet count 170,000/mm^3; prothrombin ratio 1·0; clotting time 1 min; bleeding time 1½ min. A midstream specimen of urine was normal and tests for faecal occult blood were negative. *Strep. pneumoniae* was isolated from a throat swab and the anti-streptolysin O titre was 200 units. LE cells were not found. X-rays of chest, knees and ankles were normal and a diagnosis of Schönlein–Henoch purpura was made.

The purpuric eruptions continued and she was given a 6-week course of cortisone but this did not affect the rash. After 3 months in hospital she was allowed to go home still having fresh crops of petechiae on her feet.

In 1964 she was readmitted because of a particularly severe recurrence of purpura following a bout of sinusitis. The rash was again mainly on the legs but there were a few spots on her arms and face. There were a few bruises on her legs and both legs were oedematous. BP was 95/60. Investigations on this occasion included blood counts, measurement of serum proteins and urea, examination of urine, determination of bleeding time, clotting time, one-stage prothrombin time, prothrombin consumption index, anti-haemophilic globulin assay, thromboplastin generation test, platelet counts, and X-rays of chest and sinuses. All gave normal results, but the ESR reached a maximum of 55 mm in 1 hr and β-haemolytic streptococci of group A were grown from a throat swab. She was given penicillin and discharged after 1 month. In May 1964 she was referred to Professor Harold Scarborough who confirmed the diagnosis of Schölein–Henoch disease, probably related to recurrent upper respiratory tract infections. Thereafter she continued to suffer from recurrent bouts of purpura on the legs, usually fairly closely related to upper respiratory infections.

In 1969 she was admitted because the rash had been present continuously for about 3 months. On this occasion it was not related to infection and she had not taken any drugs. She had also had pain in the knees and ankles and had noted that her hands were slightly swollen. Clinical examination revealed a very marked macular, slightly raised, erythematous rash over the front of her legs and thighs, with sparse lesions on the forearms. The rash was described as 'typical anaphylactoid purpura'. The left ankle joint was swollen and there was an effusion in the right knee joint and she was given prednisone. The rash improved within 1 month but this was thought to be unrelated to prednisone therapy and this was stopped. Since then she has continued to have purpura of the legs. Crops appear almost daily, especially after standing, but she is otherwise well and disability is minimal.

Extensive investigations during recent years have failed to reveal any urinary or haematological abnormalities. LE cells have never been found, the blood urea has always been <30 mg/100 ml and the creatinine clearance has varied between 67 and 89 ml/min. The total serum protein and electrophoresis have been normal but serum IgA concentration has been persistently raised (540 mg/100 ml; 550 mg/100 ml; 640 mg/100 ml). Cryoglobulins

were reported on one occasion but this could not be confirmed and the direct Coombs' test was negative. A skin biopsy done on 26 August 1970 showed the characteristic histological changes of anaphylactoid purpura (Dr H. J. Whiteley). The only other abnormality was the persistent absence of complement discussed below.

MATERIALS AND METHODS

Blood, taken by venepuncture, was allowed to clot at room temperature and the serum separated by centrifugation. Serum was stored at −70°C or in liquid nitrogen.

Complement assays

The techniques used for measuring total complement and its components are those described by Lachmann, Hobart & Aston (1973).

Platelet factor 3 activation test

For this test the patients' blood was taken into acid-citrate-dextrose (ACD) and centrifuged in the cold. The supernatant plasma was separated and stored at −70°C. The test was performed as described by Brown & Lachmann (1973).

Electron microscopy

One millilitre of serum was mixed with an equal quantity of phosphate buffered saline and centrifuged for ½ hr at 12,000 *g*. The supernatant from this centrifugation was discarded and the pellet resuspended in 2 ml phosphate buffered saline. This was re-centrifuged for ½ hr at 12,000 *g* and the supernatant discarded. The final pellet was resuspended in 0·1 ml distilled water. A drop of this suspension was mixed with an equal quantity of 3 per cent phosphotungstic acid adjusted to pH 6 with N KOH. A drop of this mixture was placed on a 400 mesh carbon-formvar coated grid and excess fluid withdrawn with filter paper. Immediately on drying the grid was examined in a Philips 300 Electron microscope.

RESULTS

Complement studies

Serum samples taken from the patient over a period of 18 months were studied with essentially similar results. The significant findings are shown in Table 1. The total complement measured haemolytically was about 1% normal and the haemolytic C2 level was similarly low. All other complement components measured were present in normal amounts. Capacity to generate EAC14 from EA was not substantially reduced but there was no detectable capacity to generate EAC142. The haemolytic titre could be restored to normal by the addition of functionally purified C2. These findings are all wholly compatible with a primary deficiency of C2 and are similar to those previously described.

Two other findings are of interest. The first is that the level of glycine-rich β-glycoprotein (GBG) as measured antigenically is about half the normal level. The second is that the total complement as measured by conglutination gives a titre that is not far from normal. Since the conglutination reaction requires substantial amounts of fixed C3 on the intermediate, this shows that despite the very low level of C2 the degree of fixation of C3 is not greatly impaired. This finding is consistent with the earlier reports that immune adherence

TABLE 1. Complement profile of C2 deficient patients compared with that of a normal individual*

	Total haemolytic complement			Capacity to generate		GBG
	CH50/ml	Conglutination	C2 titre	EAC14† titre	EAC142‡ titre	antigenically (%)
L.M.	11	80	4	500	0	44
Unrelated healthy C2-deficient patient	25	80	4	500	0	53
Normal human serum (NHS)	920	160	10,000	1,000	320	100

* Titres of C4, C3, C5, C6, C7 and C8+C9 were all normal. The patient's serum was not inhibitory to NHS in the C2 assay. Addition of functionally purified C2 to L.M. serum restored its haemolytic activity.

† Serum dilutions were incubated with EA at 37°C for 30 min. After washing 0·2 ml 1 : 20 guinea-pig C-NH_3 was added and a further 30-min incubation at 37°C was given.

‡ As † above but the second incubation was performed with 0·2 ml 1 : 50 guinea-pig C-EDTA.

and bactericidal activity are normal in C2-deficient sera (Gewurz *et al.*, 1966).

It is not at present clear whether the low levels of C2 detected in the haemolytic assay represent the presence of small amounts of this component or whether this low degree of activity in the assay can be brought about by factors of the alternate pathway. One of these factors, glycine-rich β-glycoprotein (which is equivalent to factor B of the properdin system), is present in somewhat reduced amounts but in the absence of turnover studies it is not known whether this represents a decrease in production or an increase in utilization.

Serum from a second C2 deficient subject, who is healthy, was kindly made available to us by Dr F. Stratton. The complement findings in this second serum were entirely similar to those of the present patient, including the low levels of GBG. It would, therefore, seem that the complement profile of our patient represents that found in C2 deficiency and is not influenced by this patient's purpura.

The capacity of ACD plasma to support endotoxin-induced damage to rabbit platelets can be used as a test of alternate pathway function (Brown & Lachmann, 1973). For example, C4-deficient guinea-pig plasma with an alternate pathway presumed to be intact, supports the platelet damaging effect of endotoxin, whereas plasma depleted of C3–9 and alternate pathway components by cobra venom factor is inactive in this respect.

Using this principle washed rabbit platelets were resuspended in the patient's platelet-poor plasma, to make reconstituted platelet rich plasma. Endotoxin (*Serratia marcescens*) was added to a final concentration of 1000 μg/ml and platelet factor 3 (PF3) activation was used as a measure of platelet damage by endotoxin. It was found that the patient's plasma supported damage (10–17% PF3 release) to the same degree as normal human plasma (5–26% PF3 release).

Electron microscopy

Examination of the negatively stained serum preparation in the electron microscope revealed numerous structures that displayed the appearance associated with mycoplasma

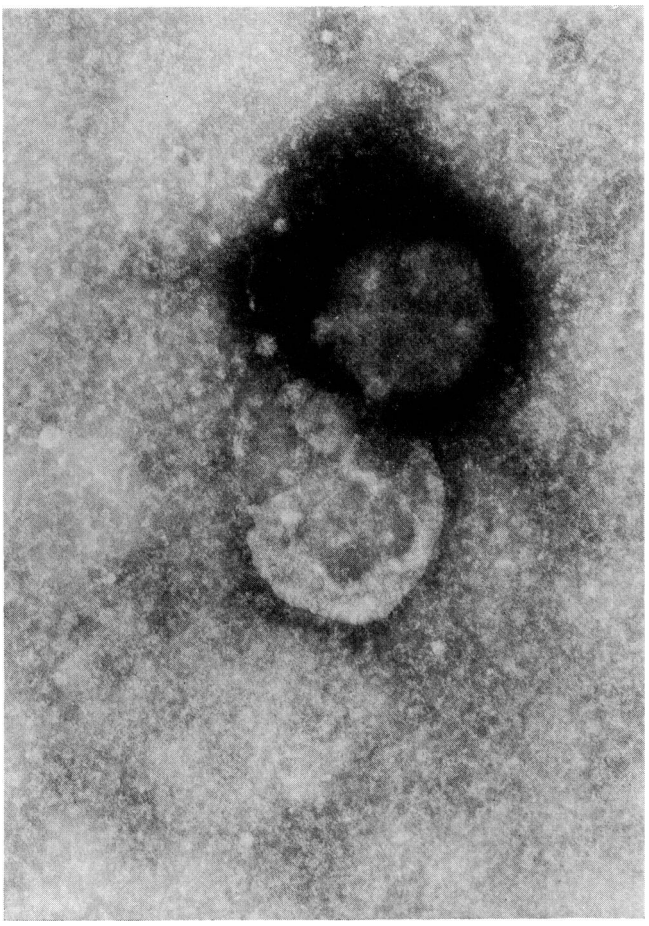

FIG. 1. Electron micrograph showing mycoplasma-like structures in patient's serum. Negative staining (× 108,000).

when prepared by the same technique (Figs 1–4). Unlike virus particles, mycoplasma do not display symmetry properties when examined by this technique.

However, they do yield certain distinctive structural features, for example, Figs 1–3 show a dense fringed body attached to a less dense membrane. Fig. 3 is known to be a mycoplasma and this appearance can be seen in most mycoplasmal preparations. Another structural feature of this organism is elongated forms displaying fine projections on the surface. Fig. 2 is one such form from the serum of our patient, while Fig. 4 is from a known mycoplasmal preparation. It has not been possible to isolate viable mycoplasma from this serum but many mycoplasmas are extremely difficult to grow *in vitro*. Although there can never be complete certainty on the basis of morphology alone the forms described do have the appearance of mycoplasma. A considerable number of sera have been examined by negative staining as negative controls in Australia antigen studies. These have served also as

controls for the present study and from these it is known that the forms described here are not to be found in normal sera.

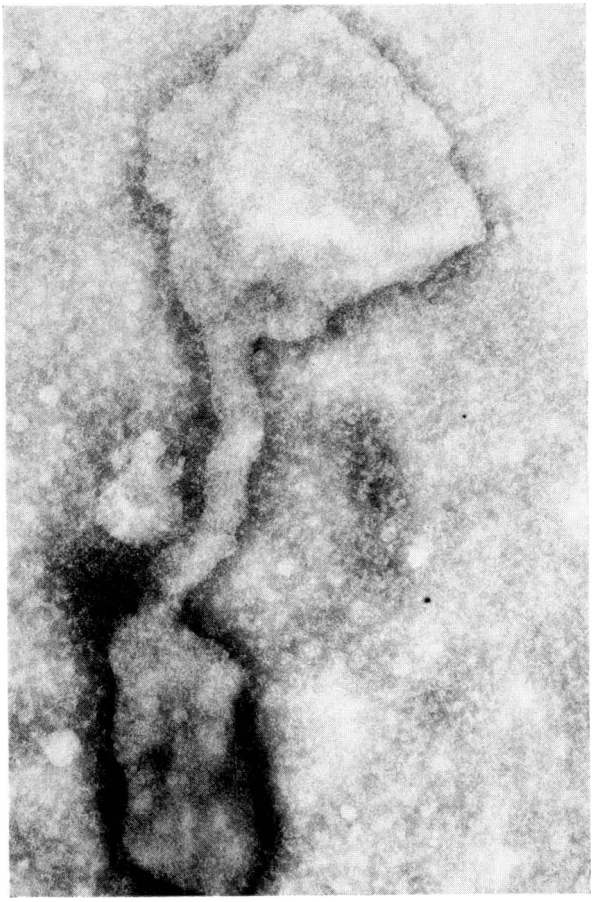

FIG. 2. Electron micrograph showing mycoplasma-like structure in patient's serum. Negative staining (× 108,000).

DISCUSSION

Isolated deficiences of the second component of complement have been described on a number of occasions in man (Klemperer *et al.*, 1966; Klemperer *et al.*, 1967; Cooper *et al.*, 1968; Ruddy *et al.*, 1970; Agnello *et al.*, 1972). The conditions is inherited as an autosomal recessive and in individuals homozygous for the deficiency the complement profile is characterized by a very low, though not entirely absent, total haemolytic complement, very low levels of haemolytic C2 and normal levels of the other classical complement components. In our patient and in the one unrelated asymptomatic C2 deficient subject whose serum we have studied there is an approximately 50 per cent reduction in the amount of glycine-rich β-glycoprotein that can be detected antigenically.

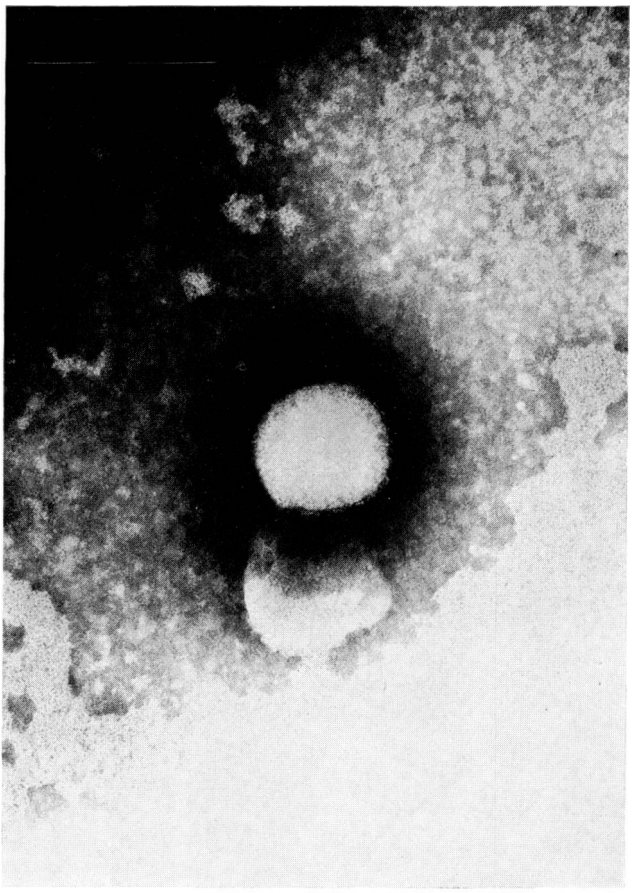

FIG. 3. Electron micrograph of control mycoplasma. Negative staining (×108,000).

In the originally described pedigrees (Silverstein, 1960; Klemperer *et al.*, 1966; Cooper *et al.*, 1968; Ruddy *et al.*, 1970) the subjects, including those homozygous for C2 deficiency, were all clinically normal. There are now, however, several examples of diseases with immunological overtones associated with C2 deficiency (Ruddy *et al.*, 1972). The patient described by Agnello *et al.* (1972) has systemic lupus erythematosus and C2 deficiency and a patient described by Pickering *et al.* (1971) has glomerulonephritis and C2 deficiency. We present here a third patient of this kind who has had an extremely prolonged history of purpura in association with C2 deficiency. The mechanism of these associations is unknown but it is unlikely to be coincidence; the rarity of C2 deficiency makes this highly unlikely (Pickering *et al.*, 1971).

It has been suggested (Agnello *et al.*, 1972) that the absence of C2 may predispose to latent virus infection. Though there is no evidence in our patient for latent or chronic virus infection mycoplasma-like structures were found in her serum. The demonstration of mycoplasma-like structures by direct electron microscopy in the serum of our patient is of

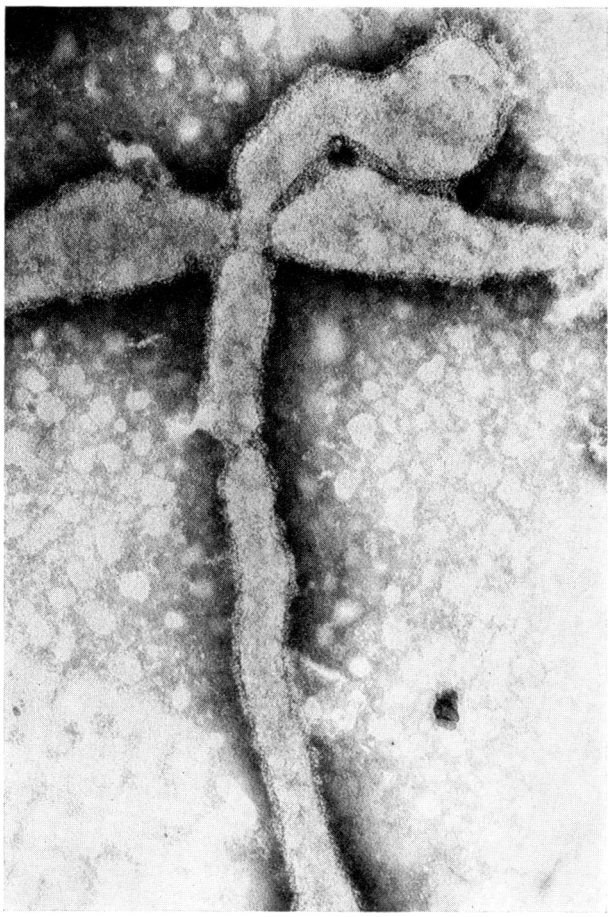

FIG. 4. Electron micrograph of control mycoplasma. Negative staining (× 108,000).

great interest though its significance in relation to C2 deficiency or the pathogenesis of the purpura are unknown. Lack of undue susceptibility to infection in C2 deficiency has been the subject of repeated comment (Silverstein, 1960; Klemperer *et al.*, 1966; Gewurz *et al.*, 1966). It is possible, however, that there are conditions in which deficiency of haemolytic complement may lead to the establishment of chronicity where lysis of the infecting agent is normally essential to terminate the infection. It is not known whether this is true of systemic mycoplasma infection.

There are at least two possible mechanisms for the pathogenesis of the purpuric rash in our patient. It could be due to chronic immune-complex disease or recurrent invasion of capillaries by an infecting agent. In the absence of alterations in complement components other than C2 there is no evidence for the former. There is, as yet, no proof for the latter possibility. It may, however, be significant that we have observed mycoplasma-like structures in the serum of one other case of persistent Schönlein–Henoch purpura.

ACKNOWLEDGMENTS

We should like to thank Dr David Brown for the Platelet Factor 3 Activation Tests and Dr D. Taylor-Robinson for his help in attempts to isolate the mycoplasma. One of us (M.S.) is indebted to the Kidney Research for Wales Foundation for providing a spectrophotometer for complement research. The financial support of the Medical Research Council to P.J.L. is also gratefully acknowledged.

REFERENCES

AGNELLO, V., DE BRACCO, M.M.E. & KUNKEL, H.G. (1972) Hereditary C2 deficiency with some manifestations of systemic lupus erythematosus. *J. Immunol.* **108,** 837.

BROWN, D. & LACHMANN, P.J. (1973) The behaviour of complement and platelets in lethal endotoxin shock in rabbits. *Int. Arch. Allergy.* (In press.)

COOPER, N.R., TEN BENSEL, R. & KOHLER, P.F. (1968) Studies of an additional kindred with hereditary deficiency of the second component of human complement (C2) and description of a new method for the quantitation of C2. *J. Immunol.* **101,** 1176.

GEWURZ, H., PICKERING, R.J., MUSCHEL, L.H., MERGENHAGEN, S.E. & GOOD, R.A. (1966) Complement-dependent biological functions in complement deficiency in man. *Lancet*, **ii,** 356.

KLEMPERER, M.R., WOODWORTH, H.C., ROSEN, F.S. & AUSTEN, K.F. (1966) Hereditary deficiency of the second component (C'2) in man. *J. clin. Invest.* **45,** 880.

KLEMPERER, M.R., AUSTEN, K.F. & ROSEN, F.S. (1967) Hereditary deficiency of the second component of complement (C'2) in man; further observations on a second kindred. *J. Immunol.* **98,** 72.

LACHMANN, P.J., HOBART, M.J. & ASTON, W.P. (1973) *Handbook of Immunology* (Ed. by D. Weir). Blackwell Scientific Publications, Oxford.

PICKERING, R.J., MICHAEL, A.F., HERDMAN, R.C., GOOD, R.A. & GEWURZ, H. (1971) The complement system in chronic glomerulonephritis. Three newly associated aberrations. *J. Pediatrics*, **87,** 30.

RUDDY, S., KLEMPERER, M.R., ROSEN, F.S., AUSTEN, K.F. & KUMATE, J. (1970) Hereditary deficiency of the second component of complement (C2) in man: correlation of C2 haemolytic activity with immunochemical measurements of C2 protein. *Immunology*, **18,** 943.

RUDDY, S., GIGLI, I. & AUSTEN, K.F. (1972) The complement system of man. *New Engl. J. Med.* **287,** 592.

SILVERSTEIN, A.M. (1960) Essential hypocomplementemia. Report of a case. *Blood*, **16,** 1338.